Supporting Information

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SI Materials and Methods

Large-Scale Overexpression of Proteins for Purification. To overexpress TM-SpoIVFB and TM-SpoIVFB E44Q, pZR236, and pZR229, respectively, were transformed into *E. coli* BL21(DE3) with selection on Luria-Bertani (LB) agar (1) containing kanamycin sulfate (50 μg/mL) or ampicillin (200 μg/mL), respectively. In each case, 10 colonies were used to inoculate 6 L HM minimal medium with yeast extract (see http://www.egr.msu.edu/pel/protocols.html) supplemented with antibiotic as above, and cultures were grown in 10-L fermentors at 30 °C. When cultures reached an optical density at 600 nm (OD_{600}) of 20, cells were induced with IPTG (250 μM) and $ZnCl_2$ (10 μM) was added. After about 4 h, when cultures reached an OD_{600} of 25, cells were collected by centrifugation (7,000 $\times$ *g* at 4 °C for 25 min) and stored at −80 °C.

To overexpress Pro-σ^K S20G, pZR327 was transformed into *E. coli* BL21(DE3) with selection on LB agar containing kanamycin sulfate (50 μg/mL). A single colony was inoculated in LB supplemented with antibiotic and the culture was incubated at 37 °C with rotation at 300 rpm until it reached an OD_{600} of 1.2. IPTG (250 μM) was added and incubation was continued for 3 h, then cells were collected by centrifugation and stored at −80 °C. Likewise, to overexpress His_{10}-CBS, pZR257 was transformed into *E. coli* BL21(DE3) and a transformant was selected, grown, and induced with IPTG.

Membrane Isolation and Solubilization. To isolate membranes containing TM-SpoIVFB or TM-SpoIVFB E44Q, cells (62 g) were resuspended and homogenized in 175 mL lysis buffer [PBS, pH 7.2 containing 100 μg/mL lysozyme, 1 μg/mL RNase A, 1 μg/mL DNase I, and complete, EDTA-free protease inhibitor mixture tablets (Roche)]. After incubation at 37 °C for 10 min, the suspension was passed twice through a French pressure cell (SLM Aminco) at 14,000 psi (96 MPa). The lysate was centrifuged (15,000 $\times$ *g* at 4 °C for 15 min) to remove cellular debris and the supernatant was centrifuged (150,000 $\times$ *g* at 4 °C for 90 min) to pellet membranes. After discarding the supernatant, the membrane pellets were resuspended in 90 mL detergent buffer [PBS, pH 7.2 containing 1% sarkosyl (Anatrace) and protease inhibitors] using a motorized dounce homogenizer, and the suspension was rotated for 60 min at 4 °C, then centrifuged (150,000 $\times$ *g* at 12 °C for 75 min). The supernatant containing detergent-solubilized membrane proteins was saved and the pellet was resuspended in detergent buffer, rotated, and centrifuged as above. The two supernatants were combined for a total of 180 mL, DM (Anatrace) was added to a final concentration of 0.5%, and the sample was rotated at room temperature for 60 min.

A similar approach was used to isolate membranes containing Pro-σ^K S20G and solubilize the protein, except a different detergent buffer [PBS, pH 7.2 containing 0.5% sarkosyl and 0.5% DDM (Anatrace)] was used. Membranes containing His_{10}-CBS were isolated and the protein was solubilized as described for Pro-σ^K S20G.

Purification of Proteins. The detergent-solubilized, His-tagged proteins were purified by cobalt-affinity chromatography followed by gel filtration chromatography. To purify TM-SpoIVFB or TM-SpoIVFB E44Q, the detergent-solubilized protein (180 mL) was added to 6 mL of Talon superflow metal affinity resin (Clontech) that had been equilibrated with buffer (PBS pH 7.2 containing 1% sarkosyl and 0.5% DM), and the mixture was rotated at room temperature for 60 min. The mixture was poured into a column, which was washed three times with 20 mL buffer (PBS, pH 7.2, 300 mM NaCl) containing different detergents (first 0.75% sarkosyl and 0.75% DM, second 0.3% sarkosyl and 0.5% DM, and third 0.3% DM). The column was eluted with a linear gradient of imidazole (0 to 150 mM) in 60 mL elution buffer (PBS, pH 7.2, 300 mM NaCl, 0.3% DM) and 1.5-mL fractions were collected, then the column was eluted with 50 mL high imidazole buffer (PBS pH 7.2, 300 mM NaCl, 0.3% DM, 300 mM imidazole). Samples were subjected to SDS/PAGE, the gel was stained with Coomassie blue, and fractions eluting at≈75 to 150 mM imidazole, which lack contaminants that elute at lower imidazole concentrations, were combined with the 300 mM high imidazole eluate. This mixture was concentrated to 4 mL using an Amicon Ultra centrifugal filter device with a 30-kDa cut-off (Millipore). The 4-mL sample was loaded onto a 1.6 cm $\times$ 60 cm Superdex 200 gel filtration column (Amersham Pharmacia) that had been equilibrated with column buffer (20 mM HEPES, pH 7.2, 150 mM NaCl, and 0.3% DM), the column was eluted with the same buffer at 0.3 mL/min, and 2-mL fractions were collected. Samples were subjected to SDS/PAGE, the gel was stained with Coomassie blue, and fractions containing purified protein were combined and concentrated to 3 mg/mL, and stored at 4 °C.

A similar approach was used to purify Pro-σ^K S20G, except the cobalt resin was equilibrated with a different buffer (PBS, pH 7.2, 300 mM NaCl, 0.2% DDM, and 10 mM imidazole) and after pouring into a column this buffer was used to wash the column. Also, the column was eluted differently, using only a high imidazole buffer (PBS, pH 7.2, 150 mM NaCl, 0.2% DDM, and 250 mM imidazole). Fractions containing Pro-σ^K S20G, as judged by SDS/PAGE followed by Coomassie blue staining, were combined and concentrated to 3 mL using a filter device with a 10-kDa cut-off. The 3-mL sample was loaded onto a 1.5 cm $\times$ 70 cm Sephadex G50 gel filtration column, eluted with buffer (PBS, pH 7.2, 150 mM NaCl, and 0.2% DDM) at 0.5 mL/min, and 2-mL fractions were collected. Fractions containing purified protein, as judged by SDS/PAGE followed by Coomassie blue staining, were combined and concentrated to 3 mg/mL, and stored at 4 °C. His_{10}-CBS was purified as described for Pro-σ^K S20G.

Reaction Mixtures for In Vitro Proteolysis with Purified Proteins. The complete reaction mixture contained purified TM-SpoIVFB or TM-SpoIVFB E44Q (2 μg) and purified Pro-σ^K S20G (4 μg) in 40 μL PBS, pH 7.2, 150 mM NaCl, 0.1% DM, 0.015% DDM, 1 mM ATP, and 6 μM zinc acetate. Some reaction mixtures contained 1, 10-phenanthroline (5 mM), GTP (1 mM), or ADP (1 mM). All reaction mixtures were incubated at 37 °C for 8 h, then terminated by the addition of 40 μL of 2$\times$ sample buffer [50 mM Tris-HCl, pH 6.8, 4% SDS, 20% (vol/vol) glycerol, 200 mM DTT, 1% sarkosyl, and 0.03% bromophenol blue] and boiling for 3 min before western blot analysis.

Reaction Mixture for ATP Binding to the Purified CBS Domain of SpoIVFB. His_{10}-CBS (10 μg) was incubated with [α-^{32}P]ATP (15 uCi) in 30 μL PBS containing 0.5 mM $MgCl_2$, 100 mM NaCl, 0.2% sarkosyl, and 5 mM imidazole at room temperature for 30 min, then 30 μL 2$\times$ sample buffer (without DTT) was added before loading samples (15 μL) onto SDS-10% polyacrylamide gels for electrophoresis followed by electroblotting to Immobilon-P membranes (Millipore).

Interaction of Pro-σ^K with the CBS Domain of SpoIVFB. To overexpress Pro-σ^K (1–126)-$FLAG_2$ and/or His_{10}-CBS, pZR262, and pZR257, respectively, were transformed into *E. coli* BL21(DE3) singly or in combination as described previously (2) and a transformant was selected, grown (500-mL culture), and induced with IPTG as described above for Pro-σ^K S20G and His_{10}-CBS. Cells were collected by centrifugation and membranes were isolated as described above except 10 mL lysis buffer containing 1 mM Pefabloc (Roche) was used to inhibit proteases rather than complete, EDTA-free protease inhibitor mixture tablets. Also, lysis buffer and all subsequent solutions contained 1 mM ATP in some experiments as indicated. Proteins were solubilized from the membrane pellets as described above except using 5 mL detergent buffer containing 1 mM PMSF rather than complete, EDTA-free protease inhibitor mixture tablets. After centrifugation, the supernatant containing detergent-solubilized membrane proteins was loaded onto a spin column containing 0.5 mL cobalt resin (Clontech) that had been equilibrated with buffer (PBS, pH 7.2, and 1% sarkosyl). The flow-through was saved for western blot analysis. The spin column was washed five times with 1 mL buffer (PBS, pH 7.2, 0.2% sarkosyl, 300 mM NaCl, and 10 mM imidazole), then eluted with 3 mL buffer (PBS, pH 7.2, 0.2% sarkosyl, 300 mM NaCl, and 300 mM imidazole). The eluate was subjected to western blot analysis.

Preparation of Liposomes. *E. coli* total lipids (120 mg) (Avanti) were suspended in 3 mL buffer (PBS, pH 7.2, 2% cholate, and 20 mM pyranine) using a motorized dounce homogenizer, followed by sonication on ice and under a stream of argon until a translucent green solution was formed. To verify that liposomes had formed, 0.5 mL was loaded onto a 1.0 cm × 50 cm Sephadex G25 gel filtration column and eluted at 1 mL/min with PBS. Liposomes that had trapped the green pyranine dye inside were eluted at the void volume of the column, whereas free dye eluted much more slowly.

Incorporation of Proteins into Liposomes. Purified protein (0.3 mg) was mixed with 1 mL preformed liposomes and rotated at 4 °C for 20 min. Detergent was removed as described (3) using Bio-Beads SM-2 adsorbent (Bio-Rad), which was added gradually (60 mg/h for the first 3 h at 4 °C and 107 mg/h for the second 3 h at 23 °C for a total of 500 mg) with continuous stirring. The resulting proteoliposomes were passed through a PD-10 column (GE Healthcare) to remove the Bio-Beads, then loaded onto a 1.2 cm × 50 cm Sephadex G50 gel filtration column and eluted at 0.6 mL/min with PBS to separate the proteoliposomes, which had trapped green pyranine dye inside and eluted at the void volume of the column, from free dye and detergent, which eluted more slowly. One-milliliter fractions were collected and the absorbance at 457 nm of green proteoliposome-containing fractions was measured, and samples were subjected to Western blot analysis. Green proteoliposome fractions (4–5 mL total) were pooled and concentrated to 2 mL using a filter device with a 30 kDa cut-off, and the concentrated proteoliposomes were stored at 4 °C.

Reaction Mixtures with Proteoliposomes. In the first type of experiment, Pro-σ^K S20G proteoliposomes (20 μL) were mixed with TM-SpoIVFB proteoliposomes (20 μL) in 50 μL (final vol) containing PBS, pH 7.2, 150 mM NaCl, 1 mM ATP, and 6 μM zinc acetate. Some reaction mixtures contained cobalt beads (10 μL) (Talon superflow metal affinity resin, Clontech). In the second type of experiment, proteoliposomes formed in the presence of both TM-SpoIVFB and Pro-σ^K S20G (40 μL) were incubated in 50 μL (final vol) as above, with some reaction mixtures containing cobalt beads (10 μL). In the third type of experiment, purified Pro-σ^K S20G (4 μg) was mixed with TM-SpoIVFB proteoliposomes (15 μL, containing ≈2 μg TM-SpoIVFB) or with TM-SpoIVFB E44Q proteoliposomes (15 μL, containing ≈2 μg TM-SpoIVFB E44Q) in 40 μL (final vol) as above. Some reaction mixtures contained 1, 10-phenanthroline (5 mM). All reaction mixtures were incubated at 37 °C for 8 h, then terminated by the addition of an equal volume (40–50 μL) of 2× sample buffer [50 mM Tris-HCl, pH 6.8, 4% SDS, 20% (vol/vol) glycerol, 200 mM DTT, 1% sarkosyl, and 0.03% bromophenol blue] and boiling for 3 min before western blot analysis.

Mass Spectrometry. After incubation, proteoliposome reaction mixtures were concentrated 5-fold using a Microcon YM-10 centrifugal filter device with a 10-kDa cut-off (Millipore). The concentrated sample (1 μL) was mixed with sinapinic acid matrix (10 mg/mL in 50% acetonitrile and 0.1% trifluoroacetic acid) at a 1:1 ratio and spotted on a stainless steel plate. Samples were analyzed on a Shimadzu Axima CFR+MALDI-TOF mass spectrometer operating in the linear mode. The ion gate was set to block ions lower than 5,000 *m*/*z*. The instrument was calibrated before sample analysis using equine cytochrome *c* and aldolase as mass standards.

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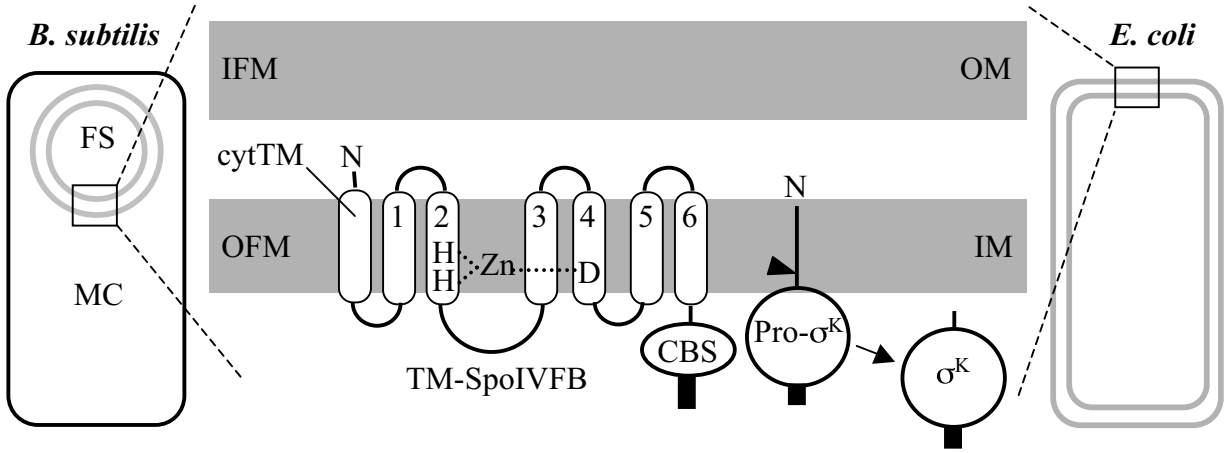

Fig. S1. Model of intramembrane proteolysis of Pro-σ^K by SpoIVFB during *B. subtilis* sporulation and when expressed in *E. coli*. Left, during endospore formation the forespore (FS) is surrounded by the IFM and the OFM, within the mother cell (MC). Middle, expanded view of the membranes surrounding the FS, or of the *E. coli* inner membrane (IM) and outer membrane (OM) in the case of heterologous expression (Right). SpoIVFB and Pro-σ^K expressed in the *B. subtilis* MC or in the *E. coli* cytosol insert into the OFM or IM, respectively. Depicted is TM-SpoIVFB, with an extra transmembrane segment (cytTM) at its N terminus. Transmembrane α-helices of SpoIVFB are numbered and membrane topology is supported by *phoA* and *lacZ* fusions (1). Histidine and aspartate residues likely to coordinate zinc are shown, as well as the C-terminal CBS domain and $FLAG_2$-His_6 tags (black rectangle). Residues 1–27 of Pro-σ^K are sufficient for membrane association (2) but whether these residues span the membrane as depicted is unknown. An arrowhead indicates the cleavage site in Pro-σ^K and a black rectangle indicates a C-terminal His_6 tag. Intramembrane cleavage of Pro-σ^K by SpoIVFB releases σ^K into the *B. subtilis* MC or into the *E. coli* cytosol.

1. Green D, Cutting S (2000) Membrane topology of the *Bacillus subtilis* Pro-σ^K processing complex. *J Bacteriol* 182:278–285.
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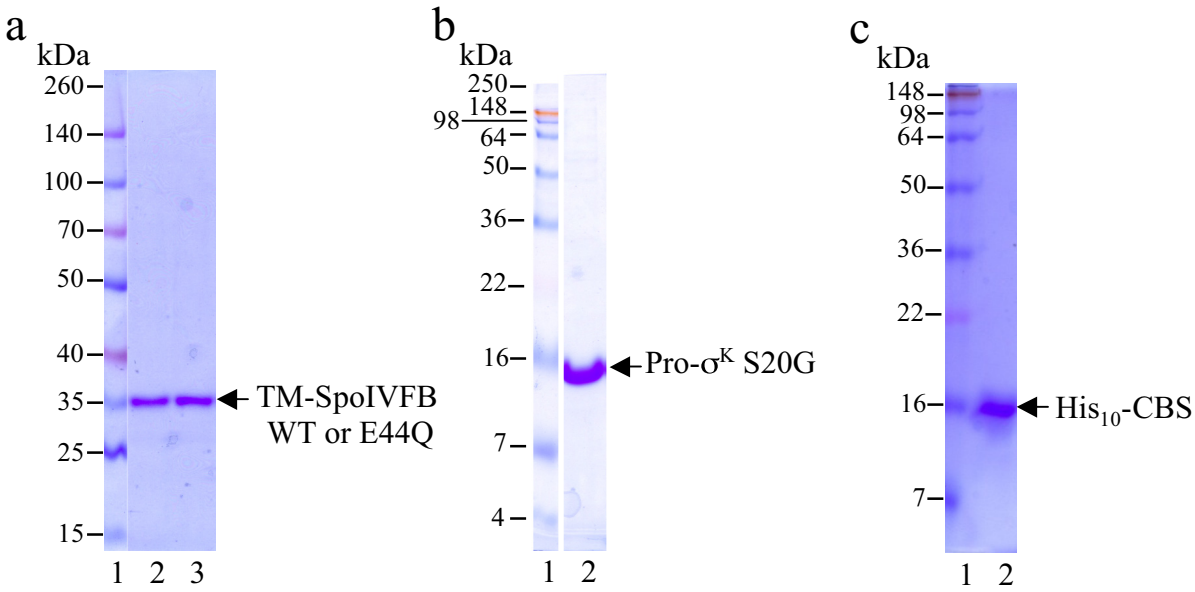

Fig. S2. Purification of recombinant proteins. (*A*) SDS/PAGE of purified TM-SpoIVFB E44Q (1 μg) (lane 2) and wild-type (WT) TM-SpoIVFB (1 μg) (lane 3) with detection by Coomassie staining. Size markers (lane 1). (*B*) SDS/PAGE of purified Pro-σ^K S20G (3 μg) (lane 2) with detection by Coomassie staining. Size markers (lane 1). (*C*) SDS/PAGE of purified His_{10}-CBS (2 μg) (lane 2) with detection by Coomassie staining. Size markers (lane 1).

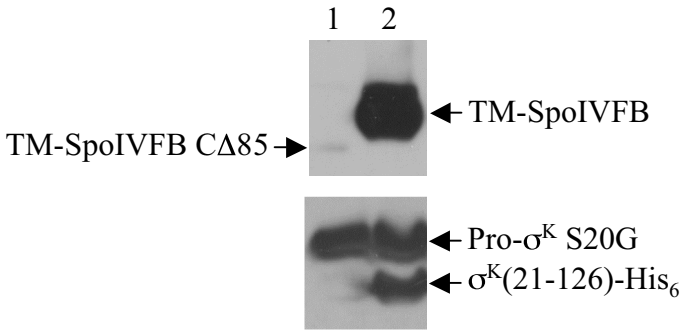

Fig. S3. The C-terminal 85 residues of SpoIVFB are important for accumulation and for cleavage of Pro-σ^K. *E. coli* cells bearing pZR327 to produce Pro-σ^K S20G and either pZR265 to produce mutant TM-SpoIVFB lacking the C-terminal 85 residues (CΔ85) (lane 1) or pZR209 to produce wild-type TM-SpoIVFB (lane 2), were collected 2 h after IPTG induction. Extracts from equivalent cell amounts were immunoblotted using antibodies against FLAG (top panel) or penta-His (bottom panel).

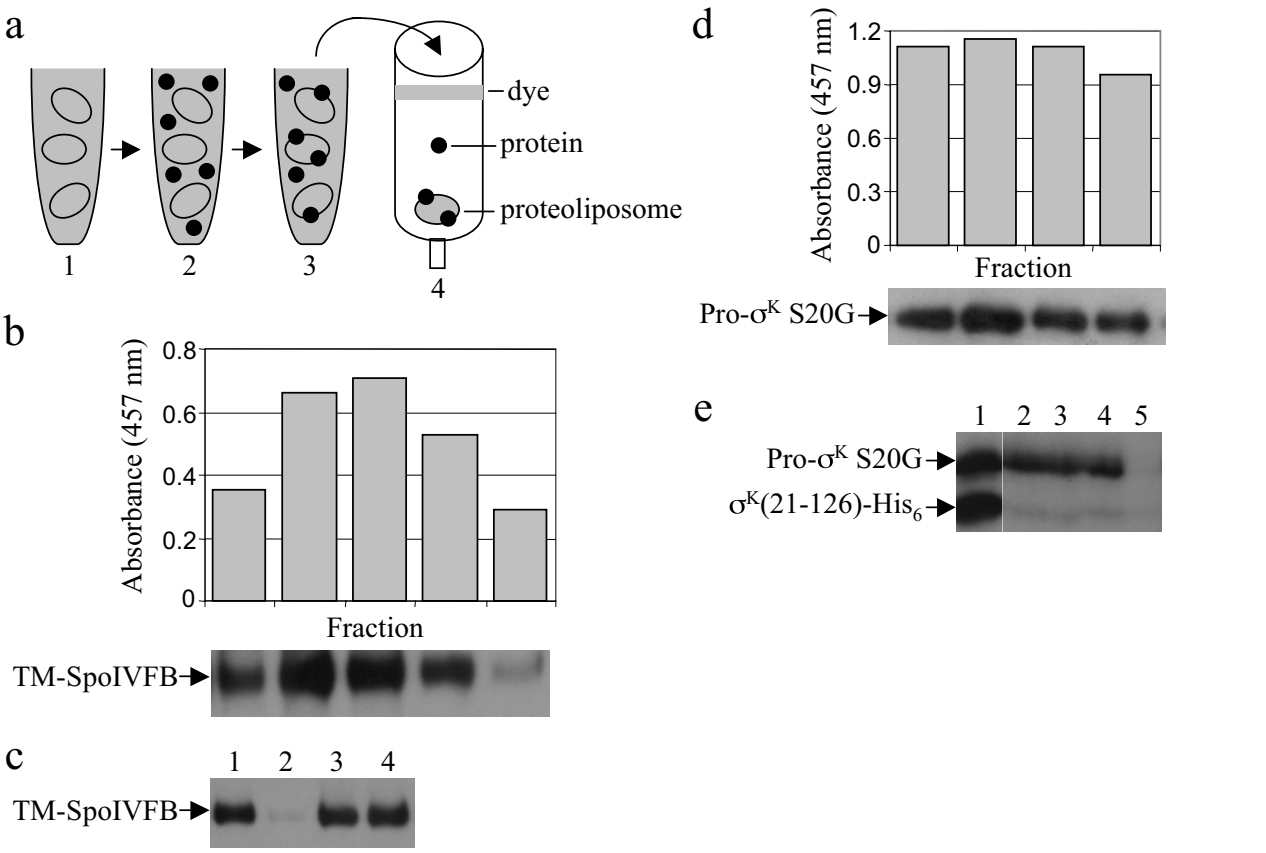

Fig. S4. Incorporation of TM-SpoIVFB and Pro-σ^K S20G into preformed liposomes. (*A*) Steps in proteoliposome formation. In step 1, *E. coli* lipids form liposomes after sonication in the presence of cholate and green pyranine dye (gray). Detergent-solubilized purified protein (black circle) is added (step 2). Protein inserts into lipid bilayer of liposomes and detergent is removed by addition of Bio-Beads SM-2 (step 3). Dye-filled proteoliposomes are separated from protein and dye by gel filtration chromatography (step 4). (*B*) Analysis of TM-SpoIVFB proteoliposomes. The graph shows absorbance of green fractions (due to pyranine trapped inside liposomes) eluted at the void volume of the gel filtration column, and the panel below shows immunoblots of the same fractions using antibodies against FLAG to detect TM-SpoIVFB. (*C*) Sedimentation of TM-SpoIVFB proteoliposomes and solubilization by detergent. TM-SpoIVFB proteoliposomes with no addition (lanes 1 and 2) or addition of sarkosyl (1%) (lanes 3 and 4) were rotated at room temperature for 20 min. Small samples were taken (lanes 1 and 3) before centrifugation (200,000 × *g* at 12 °C for 90 min) and the supernatants after centrifugation were also sampled (lanes 2 and 4). Samples were immunoblotted using antibodies against FLAG. (*D*) Analysis of Pro-σ^K S20G proteoliposomes. As in *b* except using antibodies against penta-His to detect Pro-σ^K S20G. (*E*) Incorporation of Pro-σ^K S20G but not σ^K (21–126)-His_6 into liposomes. A mixture of Pro-σ^K S20G and σ^K (21–126)-His_6 was added to preformed liposomes (step 2 in *A*) and after detergent removal (step 3 in *A*) a small sample was taken for immunoblots using antibodies against penta-His (lane 1). The rest of the sample was subjected to gel filtration chromatography (step 4 in *A*) and the green proteoliposome fractions were pooled. A small sample was taken (lane 2) and the rest was centrifuged (200,000 × *g* at 12 °C for 90 min). The supernatant was saved (lane 5). The green pellet (due to pyranine trapped in liposomes) was resuspended in an equal volume and a small sample was taken (lane 3) before addition of DDM (2%) at room temperature for 20 min with rotation, followed by centrifugation (200,000 × *g* at 12 °C for 90 min). The green supernatant (due to pyranine released from detergent-solubilized liposomes) (lane 4) and the other samples were immunoblotted using antibodies against penta-His.

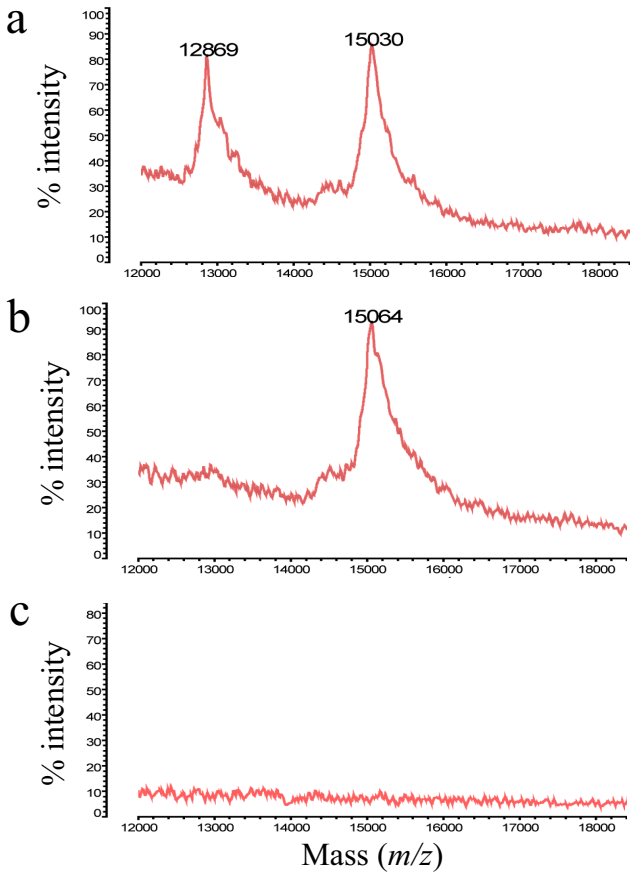

Fig. S5. Cleavage of Pro-σ^K S20G is accurate. (*A*) Wild-type TM-SpoIVFB proteoliposomes incubated with Pro-σ^K S20G. (*B*) Mutant TM-SpoIVFB E44Q proteoliposomes incubated with Pro-σ^K S20G. (*C*) Wild-type TM-SpoIVFB proteoliposomes incubated alone in the absence of substrate. Reactions were analyzed by MALDI-TOF mass spectrometry in the mass range from *m/z* 12,000–18,000. Numbers above peaks give the mass (in *m/z*) at peak maxima.

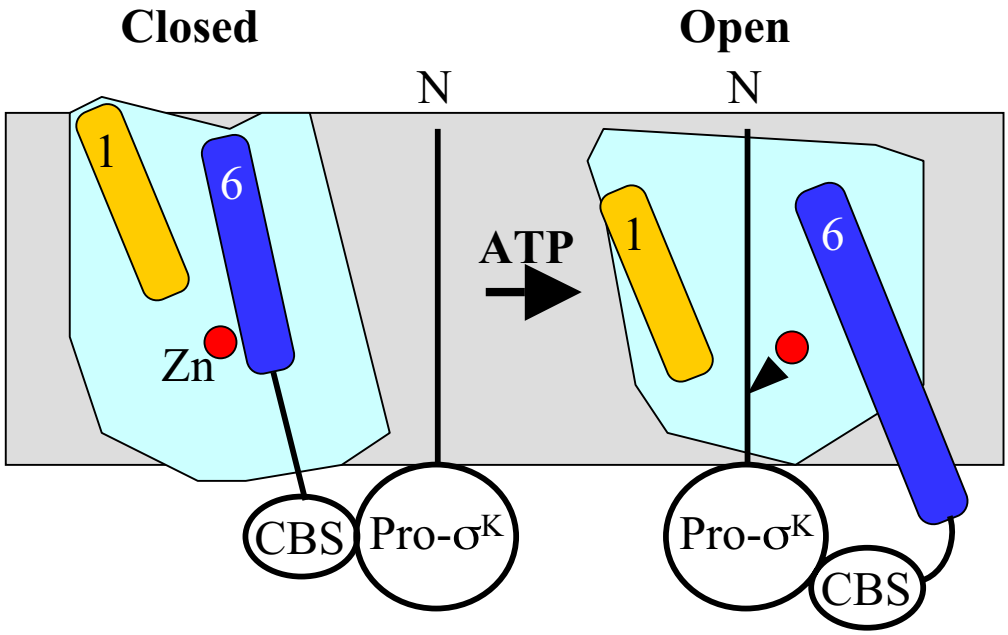

Fig. S6. Model for the role of ATP and the CBS domain of SpoIVFB in cleavage of Pro-σ^K. Based on the finding that mjS2P crystallized in closed and open conformations, a general model was proposed in which substrate access to the active site is gated by two transmembrane segments [Feng L, et al. (2007) *Science* 318:1608–1612]. In the absence of ATP, SpoIVFB (light blue) is proposed to be in a closed conformation with transmembrane segments 1 (orange) and 6 (blue) preventing access of Pro-σ^K (associated with the CBS domain of SpoIVFB) to the active site Zn atom (red). Binding of ATP to the CBS domain is proposed to change its interaction with Pro-σ^K, switching SpoIVFB to an open conformation in which transmembrane segments 1 and 6 are farther apart and allow entry of the N-terminal pro-sequence of Pro-σ^K for cleavage (arrowhead). The proposed outline of SpoIVFB and the positions of transmembrane segments 1 and 6, and Zn, are based on the structures of mjS2P in closed and open conformations [Feng L, et al. (2007) *Science* 318:1608–1612].

Table S1. Plasmids used in this study

Plasmid	Description	Construction	Ref.
pZR12	Km^R; T7-Pro-σ^K(1–126)-H6		1
pLW01	Ap^R; T7		2
pSL28a	Ap^R; T7-H10-SpoIVFB(197–288)		3
pRMG-cytTM-F-H6	Ap^R; T7-cytTM-TEV-F-H6	Coding sequences for cytTM and a TEV protease cleavage site were cloned between the NcoI and BamHI sites of the pLW01 MCS and coding sequences for F and H6 were cloned between the HindIII and XhoI sites of the pLW01 MCS	This study
pZR91	Ap^R Km^R; Pro-σ^K(1–126)	Part of *sigK* (coding for residues 1–126) was amplified by PCR using pZR12 as template and primers LK1032 and LK1033, then cloned into pCR2.1-TOPO (Invitrogen)	This study
pZR131	Ap^R; T7-cytTM-SpoIVFB	*spoIVFB* was amplified by PCR using *B. subtilis* PY79 (4) chromosomal DNA as template and primers LK1385 and LK1386, then cloned into BamHI-HindIII-digested pRMG-cytTM-F-H6	This study
pZR200	Ap^R; T7-F2-H6	Oligonucleotides LK1865 and LK1866 were annealed, forming the coding sequence for F2, which was cloned into HindIII-XhoI-digested pET21b (Novagen)	This study
pZR209	Ap^R; T7-cytTM-SpoIVFB-F2-H6	XbaI-HindIII fragment from pZR131 was cloned into XbaI-HindIII-digested pZR200	This study
pZR229	Ap^R; T7-cytTM-SpoIVFB E44Q-F2-H6	pZR209 was subjected to site-directed mutagenesis using primers LK245 and LK246 to make an E44Q substitution in SpoIVFB	This study
pZR236	Km^R; T7-cytTM-SpoIVFB-F2-H6	XbaI-XhoI fragment from pZR209 was cloned into XbaI-XhoI-digested pET29b (Novagen)	This study
pZR244	Ap^R; T7-cytTM-SpoIVFB CΔ10-F2-H6	pZR209 was subjected to site-directed mutagenesis using primers LK2071 and LK2072 to delete sequences coding for the C-terminal 10 residues of SpoIVFB	This study
pZR245	Ap^R; T7-Pro-σ^K(1–126)-F2-H6	NdeI-SalI fragment from pZR91 was cloned into NdeI-SalI-digested pZR200	This study
pZR256	Ap^R; T7-cytTM-SpoIVFB(SalI)-F2-H6	pZR209 was subjected to site-directed mutagenesis using primers LK2089 and LK2090 to create a SalI site after the codon for residue 204 of SpoIVFB	This study
pZR257	Km^R; T7-H10-SpoIVFB(197–288)	XbaI-BamHI fragment from pSL28a was cloned into XbaI-BamHI-digested pET29b (Novagen)	This study
pZR260	Ap^R; T7-SpoIVFB-F2-H6	pZR209 was subjected to site-directed mutagenesis using primers LK2110 and LK2111 to delete sequences coding for cytTM	This study
pZR262	Ap^R; T7-Pro-σ^K(1–126)-F2	BglII-XhoI fragment from pZR245 was cloned into BglII-XhoI-digested pET16b (Novagen)	This study
pZR265	Ap^R; T7-cytTM-SpoIVFB CΔ85-F2-H6	XbaI-SalI fragment from pZR256 was cloned into XbaI-SalI-digested pZR200	This study
pZR327	Km^R; T7-Pro-σ^K(1–126)-H6 S20G	pZR12 was subjected to site-directed mutagenesis using primers LK2270 and LK2271 to make an S20G substitution in Pro-σ^K(1–126)-H6	This study

Abbreviations: Km^R, kanamycin-resistant; T7, T7 RNA polymerase promoter and a translation initiation sequence; H6, six histidine residues; Ap^R, ampicillin-resistant; H10, ten histidine residues; cytTM, transmembrane segment from rabbit Cytochrome P450 2B4; TEV, Tobacco Etch Virus protease cleavage site; F, one FLAG epitope; MCS, multiple cloning site; F2, two FLAG epitopes.

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4. Youngman P, Perkins JB, Losick R (1984) Construction of a cloning site near one end of Tn*917* into which foreign DNA may be inserted without affecting transposition in *Bacillus subtilis* or expression of the transposon-borne *erm* gene. *Plasmid* 12:1–9.

Table S2. Oligonucleotides used in this study

Oligonucleotide	Sequence*
LK245	5′-gattgtattgattcatCagctggggcatgctgc
LK246	5′-gcagcatgccccagctGatgaatcaatacaatc
LK1032	5′-gaAGATCTcatatggtgacaggtgttttcgc
LK1033	5′-gcGTCGACgcaacagccaggacaacaggatccttttttttgttttttt
LK1385	5′-cgGGATCCatgaataaatggctcgaccttatc
LK1386	5′-cccAAGCTTgtagggcagaagcagttcctcca
LK1865	5′-AGCTTgattacaaggatgacgatgacaaggattacaaggatgacgatgacaagc
LK1866	5′-TCGAGcttgtcatcgtcatccttgtaatccttgtcatcgtcatccttgtaatca
LK2071	5′-gataagcggacgaataagcttgattacaag
LK2072	5′-cttgtaatcaagcttattcgtccgcttatc
LK2089	5′-cggcaaaggcactatGTCGACgtgagatttctcc
LK2090	5′-ggagaaatctcacGTCGACatagtgcctttgccg
LK2110	5′-gaaggagatataccatgaataaatggctcg
LK2111	5′-cgagccatttattcatggtatatctccttc
LK2270	5′-gtctttttagtaGGTtacgtgaaaaac
LK2271	5′-gtttttcacgtaACCtactaaaaagac

*Capital letters indicate a mutation or a restriction site.